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(54) Recombinant polypeptides and their uses, including assay for AIDS virus.

(57) The HIV portions of 4 proteins are useful for the diagnosis of, and in preparing vaccines for, the HIV virus.

EP 0 311 228 A2

RECOMBINANT POLYPEPTIDES AND THEIR USES, INCLUDING ASSAY FOR AIDS VIRUS

This invention relates to recombinant polypeptides and their uses, including assay for AIDS virus. A full background, and polypeptides which can be used for the same purpose, are given in EP-A-0255190, published 03.02.88.

5 In particular, EP-A-0255190 discloses proteins identified as R10, PB1, 590 and KH1. Their amino-acid sequences are given, as are the nucleotide sequences encoding these fusion proteins.

Novel proteins according to the present invention are the HIV portions of proteins R10, PB1, 590 and KH1, the amino-acid sequences of which are respectively given in claims 1, 2, 3 and 4, and also those sequences without the N-terminal methionine. A further aspect of the invention comprises DNA encoding
10 the novel proteins, (examples of such nucleotide sequences are shown in the accompanying Figures 1, 2, 3 and 4 respectively) and transfer vectors comprising such DNA, e.g. plasmids pΔPB1 and pΔ2PB1.

The novel proteins can be used in assays for detecting or quantifying antibody against HIV, e.g. when attached to an immunoabsorbent solid phase such as is provided by a glass or plastic bead, a well of a microtitre plate or a test tube. The proteins may also be incorporated into vaccine compositions, as is also
15 generally described in EP-A-0255190.

The following illustrates the present invention.

Non-fusion Derivative of PB1

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A non-fusion derivative of the PB1 protein, containing no non-HIV amino-acids other than an N-terminal methionine, was constructed using oligonucleotide-directed site-specific mutagenesis (Inouye, S. and Inouye, M., "Synthesis & Applications of DNA & RNA", ed. Narang, Saran A. Academic Press, 1987). In this
25 procedure, 90 non-HTLV-III bp upstream and 39 downstream of the env gene sequence in pPB1 were deleted via DNA loopouts generated by hybridization with synthetic oligonucleotides.

The oligonucleotide synthesized for the N-terminal loopout was designed so that the start codon of the β -glucuronidase gene is placed immediately adjacent to the 5' end of the HTLV-III env gene sequence (Figure 5). The oligonucleotide includes sequences homologous to both sides of this newly created junction
30 that allow proper hybridization to the plasmid DNA.

The two DNA molecules used to form a heteroduplex with a single-stranded gap that is the substrate for hybridization were created by digesting pPB1 with Sall and HpaI, or with PstI linearized pPB1, and a double digest with Sall and HpaI yields fragments of 3800 and 700 bp, the larger of which was gel-isolated for use in the mutagenesis.

35 Kinasing of the oligonucleotide, hybridization, polymerization and ligation to yield closed circular molecules were done according to the methods of Inouye and Inouye mentioned above. To enrich for DNA molecules containing the deletion, the DNA mixture was digested with MluI, which cuts within the region being deleted.

The digested DNA was used to transform competent E. coli JM105 cells and plasmid-containing
40 transformants were isolated by overnight growth on YT (8 g tryptone, 5 g yeast extract, and 5 g NaCl per liter) Cm plates at 37° C.

Plasmid DNA was isolated from each transformant and screened for the correct construction by simultaneous digestion with MluI and HindIII. Molecules that were not deleted yielded fragments of approximately 3900 and 600 bp. Those containing the deletion did not have the MluI site and yielded linear
45 molecules of approximately 4400 bp. Plasmid DNA from transformants that appeared to contain the deletion was retransformed to ensure segregation of deleted and non-deleted plasmids and the recovery of pure plasmid populations. DNA from these second transformants was analyzed as in the previous digest and was determined to have the correct construction. This plasmid was designated pΔPB1.

To eliminate the C-terminal non-HTLV-III amino acids, oligonucleotide-directed site-specific mutagenesis
50 was carried out as above, using the pΔPB1 plasmid as a substrate. The oligonucleotide (Figure 6) was designed to position the TGA codon that occurs out-of-frame downstream from the env gene sequence so that it is immediately adjacent to the 3' end of the env gene sequence and in-frame to act as a translational stop codon.

The molecules to form the heteroduplex used for hybridization were created by digesting pΔPB1 and PstI alone or with KpnI and HpaI. The large KpnI/HpaI fragment encompassing most of the vector was gel-

isolated for use in the mutagenesis. Kinasing, hybridization, polymerization and ligation were performed as above. Enrichment for deleted molecules was accomplished by digesting with HindIII, which cuts within the region being deleted. The DNA was used to transform cells as above.

Plasmid DNA was isolated from each transformant and screened for the correct construction by simultaneous digestion with EcoRI and HpaI. The deleted plasmid yields two restriction fragments of 2900 and 1750 bp. Plasmid DNA showing this pattern was retransformed as above, and DNA from these transformants was analyzed with the same digest. This plasmid, containing N-terminal and C-terminal deletions, is designated pd2PB1.

When the strain harboring plasmid pΔPB1 is grown in 2% medium (2% yeast extract, bactotryptone, casamino acids [Difco, Detroit, MI], 0.2% potassium monobasic, 0.2% potassium dibasic, and 0.2% sodium dibasic) containing 50 µg/ml ampicillin and the total complement of cellular proteins electrophoresed on an SDS-polyacrylamide gel, a protein of approximately 22 kD can be visualized by either coomassie blue staining or by western blot analysis using as probe selected sera from animals immunized with recombinant env gene proteins. Under the same conditions, a protein of approximately 20 kD is produced in a strain containing pd2PB1.

The technique of oligonucleotide-directed site-specific mutagenesis can be used in a similar way to eliminate the non-HTLV-III amino acids flanking the env gene fusion proteins R10, 590, and KH1.

In the procedure detailed above, the removal of the non-HTLV-III sequence from the fusion proteins involves removal of amino acids at both the N-terminus and the C-terminus of the protein and is accomplished in two sequential steps.

It is well known in the art that a methionine at the N-terminal position can be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from *E. coli* (Ben-Bassat, A., Bauer, K., Chang, S.-Y., Myambo, K., Boosman, A. and Chang, S. [1987] *Journal of Bacteriology* 169(2): 751-757) and *Salmonella typhimurium*, and in vitro activity has been demonstrated on recombinant proteins (Miller, C.G., Strauch, K.L., Kukral, A.M., Miller, J.L., Wingfield, P.T., Massei, G.J., Werlen, R.C., Graber, P. and Movva, N.R. [1987] *Proc. Natl. Acad. Sci. USA* 84:2718-2722). Therefore, removal of an N-terminal methionine may be achieved either in vivo by expressing the protein in a host which produces MAP (e.g., *E. coli* CM89 or *S. cerevisiae*), or in vitro by use of purified MAP (e.g., procedure of Miller et al.).

pd2PB1 Purification

Unless specified otherwise, all steps are carried out at room temperature.

Lysis--Three 700 ml bottles of frozen cell paste containing pd2PB1 are thawed at 37° C, and are then spun at 4,000 rpm in a J-6B centrifuge with a JS-4.2 rotor (Beckman, Palo Alto, CA) at 4° C for 30 min. The supernatant is then discarded and the weight of the cell pellet is determined. The cell pellet (typically 1 kg) is resuspended in 2 volumes of lysis buffer (v/w) which consists of 8 M urea, 20 mM Tris-HCl (pH 7.5 ± 0.1), 1 mM EDTA, 14.7 mM 2-mercaptoethanol and 1 mM PMSF.

The resuspended cell pellet is run through a Type TDK Pilot DYNO-MILL® (Impandex Inc., Maywood, NJ) containing 0.5-0.7 mm glass beads at 200-400 ml/min. Prior to use the DYNO-MILL® is charged with one liter of lysis buffer and cooled so that the solution flowing through is at less than ambient temperature. The resuspended cell pellet is passed through the DYNO-MILL® twice, and after the second pass, the DYNO-MILL® is washed with 1 liter of lysis buffer. Lysed cell suspension and wash are pooled.

Concentration and filtration

The lysed cell suspension plus one liter wash is concentrated to 800 ml using a 0.45 µm. DURAPORE Pellicon cassette in a Pellicon 4 GPM system (Millipore, Bedford, MA). The concentration is done with an inlet pressure of less than or equal to 275 kPa and an outlet pressure between 70 and 140 kPa. After concentration the lysed cell suspension is filtered with 4 liters of lysis buffer using the same Pellicon system, cassette and pressure settings with the tubing rigged for diafiltration.

Extraction

The washed lysis cell suspension is extracted with 10 l of extraction buffer consisting of 6 M guanidine

HCl, 100 mM Tris-HCl (pH 7.6 ± 0.1), and 10 mM EDTA, using the same Pellicon system, cassette and pressure settings as described above with the tubing rigged for diafiltration.

5 Buffer exchange

The filtrate from the previous step is typically concentrated to 1 liter using a Pellicon 4GPM system with two PTGC cassettes (10,000 NMWL). The concentration is done with an inlet pressure of less than or equal to 345 kPa and an outlet pressure between 205 and 310 kPa. After concentration, the supernatant is buffer
 10 exchanged with CM column buffer consisting of 8 M urea, 25 mM potassium phosphate, and 1 mM EDTA (pH 6.8 ± 0.1), with conductivity less than or equal to 3.0 ms/cm. For buffer exchange, the same Pellicon system, the same cassettes and the same pressure settings as above are used with the tubing rigged for diafiltration. Eight liters of CM column buffer are used to buffer exchange 1 liter of concentrated extract. After buffer exchange, the buffer-exchanged extract is drained from the system and the system is washed
 15 with 1 liter of CM column buffer. the buffer-exchanged extract and the wash are pooled and the solution's conductivity and pH are measured. The conductivity of the solution is adjusted to less than or equal to 3.0 ms/cm with deionized 8 M urea and the pH is adjusted to be within the range of 6.5-7.0.

20 CM chromatography

A 50 x 51 cm column of CM SEPHAROSE® FAST FLOW (Pharmacia, Piscataway, NJ) is equilibrated by washing the column sequentially with 4 column volumes of 0.5 M NaOH, 2 column volumes of deionized water and 2-3 column volumes of CM column buffer. The column is considered equilibrated when the pH of
 25 the outflow is within 0.2 units of the CM column buffer and the conductivity of the outflow is within 0.3 ms/cm of the CM column buffer.

For loading, the buffer exchanged extract is pumped on to the column at an inlet pressure between 10 and 15 psi. After loading, the CM column is washed with CM column buffer until the OD at 280 nm of the outflow is less than 0.1. The pd2PB1 is then eluted with an 8-liter linear gradient of 0-0.5 M NaCl in CM
 30 column buffer and collected in 100 ml fractions. The fractions are assayed by SDS-PAGE and Western with anti-gp160 antibody, and those containing significant pd2PB1 and trace contaminants are pooled.

Organic extraction

35 The pooled protein solution from the previous step is brought to a ratio of 55% acetonitrile to 45% protein solution (v/v) by the slow addition of pure acetonitrile with mixing. After addition of all of the acetonitrile, the solution is centrifuged in a J2-21 centrifuge using a JA10 rotor (Beckman) at 10,000 rpm and 4 °C for 15 min. After centrifugation, the supernatant is collected and the pellet is discarded.

40 The centrifugation supernatant is brought to a ratio of 35% ethanol to 65% supernatant (v/v) by slow addition of 95% ethanol with mixing. After addition of all of the ethanol, the solution is centrifuged in a J2-21 centrifuge using a JA-10 rotor at 10,000 rpm and 4 °C for 15 min. After centrifugation the pellet is collected and the supernatant is discarded.

45 The pellet is allowed to air dry for 15 min, and is then redissolved in S-300 column buffer, which consists of 8 M urea, 0.3 M glycine, 5 mM EDTA, 15 mM 2-mercaptoethanol, 1 mM dithiothreitol (DTT) (pH 8.50 ± 0.01). The pellet is dissolved in a volume of S-300 column buffer equal to one-tenth the volume of the pooled protein solution at the beginning of this step.

50 Concentration

The absorbance of the redissolved protein solution from above is determined at 280 nm and an approximate protein concentration is determined by assuming that a 1 mg/ml solution of protein has an absorbance of 1.0 at 280 nm. The solution is concentrated to 10 mg/ml using a 200 ml Amicon stirred cell
 55 concentrator with a YM-10 membrane.

S-300 chromatography

Thirty to seventy ml of the concentrated protein solution is loaded on a 5.0 x 135 cm column of SEPHACRYL® S-300 from Pharmacia. The column had been previously equilibrated with S-300 column buffer which consists of 8 M urea, 0.3 M glycine, 5 mM EDTA, 15 mM 2-mercaptoethanol, 1 mM DTT (pH 8.50 ± 0.01). After loading, the column is run isocratically in the same buffer. Twenty ml fractions are collected and the fractions are assayed for pd2PB1 content by SDS-PAGE.

Equal volume aliquots are taken from suitable fractions containing pd2PB1 and are used to determine which fractions are satisfactory for pooling. The aliquots are pooled, dialyzed overnight versus 8 M urea, 25 mM sodium phosphate, 1 mM EDTA (pH 6.8 ± 0.1), and the OD at 280 nm of the dialyzed pool is determined using the dialysis buffer as blank. The protein concentration of the solution is determined using the calculated extinction coefficient of pd2PB1 of 1.0 (mg/ml)⁻¹. SDS-PAGE is run on 10 µg of the dialyzed pooling using a 15% SDS acrylamide gel. After coomassie staining and destaining, the gel is scanned using an LKB (Gaithersburg, MD) scanning densitometer attached to a Waters (Milford, MA) 740 Integrator. If the pd2PB1 band on the gel is more than 97% pure, then the fractions that were used for the aliquot are checked for endotoxins at a 1 to 20 dilution in the Limulus Amebocyte Lysate (LAL) assay using 0.06 eu/ml tubes. If the LAL test on the diluted fractions is negative, the fractions are pooled and used for subsequent operations. If the gel fails to meet the purity specification, the process is repeated using equal volume aliquots from a different set of fractions. Only those fractions having a negative LAL test at a 1 to 20 dilution are pooled.

Claims

1. A protein having the amino-acid sequence

5 MetValTrpLysGluAlaThrThrThrLeuPheCysAlaSerAspAlaLysAlaTyr
 AspThrGluValHisAsnValTrpAlaThrHisAlaCysValProThrAspPro
 10 AsnProGlnGluValValLeuValAsnValThrGluAsnPheAsnMetTrpLys
 AsnAspMetValGluGlnMetHisGluAspIleIleSerLeuTrpAspGlnSer
 LeuLysProCysValLysLeuThrProLeuCysValSerLeuLysCysThrAsp
 15 LeuLysAsnAspThrAsnThrAsnSerSerSerGlyArgMetIleMetGluLys
 GlyGluIleLysAsnCysSerPheAsnIleSerThrSerIleArgGlyLysVal
 20 GlnLysGluTyrAlaPhePheTyrLysLeuAspIleIleProIleAspAsnAsp
 ThrThrSerTyrThrLeuThrSerCysAsnThrSerValIleThrGlnAlaCys
 ProLysValSerPheGluProIleProIleHisTyrCysAlaProAlaGlyPhe
 25 AlaIleLeuLysCysAsnAsnLysThrPheAsnGlyThrGlyProCysThrAsn
 ValSerThrValGlnCysThrHisGlyIleArgProValValSerThrGlnLeu
 30 LeuLeuAsnGlySerLeuAlaGluGluGluValValIleArgSerAlaAsnPhe
 ThrAspAsnAlaLysThrIleIleValGlnLeuAsnGlnSerValGluIleAsn
 CysThrArgProAsnAsnAsnThrArgLysSerIleArgIleGlnArgGlyPro
 35 GlyArgAlaPheValThrIleGlyLysIleGlyAsnMetArgGlnAlaHisCys
 AsnIleSerArgAlaLysTrpAsnAsnThrLeuLysGlnIleAspSerLysLeu
 40 ArgGluGlnPheGlyAsnAsnLysThrIleIlePheLysGlnSerSerGlyGly
 AspProGluIleValThrHisSerPheAsnCysGlyGlyGluPhePheTyrCys
 AsnSerThrGlnLeuPheAsnSerThrTrpPheAsnSerThrTrpSerThrLys
 45 GlySerAsnAsnThrGluGlySerAspThrIleThrLeuProCysArgIleLys
 GlnIleIleAsnMetTrpGlnGluValGlyLysAlaMetTyrAlaProProIle
 50 SerGlyGlnIleArgCysSerSerAsnIleThrGlyLeuLeuLeuThrArgAsp
 GlyGlyAsnSerAsnAsnGluSer

2. A protein having the amino-acid sequence

Met LeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLys
SerIleArgIleGlnArgGlyProGlyArgAlaPheValThrIleGlyLysIle
5 GlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAsnThr
LeuLysGlnIleAspSerLysLeuArgGluGlnPheGlyAsnAsnLysThrIle
10 IlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsn
CysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSerThrTrp
PheAsnSerThrTrpSerThrLysGlySerAsnAsnThrGluGlySerAspThr
15 IleThrLeuProCysArgIleLysGlnIleIleAsnMetTrpGlnGluValGly
LysAlaMetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsnIle
20 ThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsnAsnGluSer

3. A protein having the amino-acid sequence

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MetLeuAsnGlnSerValGluIleAsnCysThrArgProAsnAsnAsnThrArgLys
 SerIleArgIleGlnArgGlyProGlyArgAlaPheValThrIleGlyLysIle
 5 GlyAsnMetArgGlnAlaHisCysAsnIleSerArgAlaLysTrpAsnAsnThr
 LeuLysGlnIleAspSerLysLeuArgGluGlnPheGlyAsnAsnLysThrIle
 10 IlePheLysGlnSerSerGlyGlyAspProGluIleValThrHisSerPheAsn
 CysGlyGlyGluPhePheTyrCysAsnSerThrGlnLeuPheAsnSerThrTrp
 PheAsnSerThrTrpSerThrLysGlySerAsnAsnThrGluGlySerAspThr
 15 IleThrLeuProCysArgIleLysGlnIleIleAsnMetTrpGlnGluValGly
 LysAlaMetTyrAlaProProIleSerGlyGlnIleArgCysSerSerAsnIle
 20 ThrGlyLeuLeuLeuThrArgAspGlyGlyAsnSerAsnAsnGluSerGluIle
 PheArgProGlyGlyGlyAspMetArgAspAsnTrpArgSerGluLeuTyrLys
 TyrLysValValLysIleGluProLeuGlyValAlaProThrLysAlaLysArg
 25 ArgValValGlnArgGluLysArgAlaValGlyIleGlyAlaLeuPheLeuGly
 PheLeuGlyAlaAlaGlySerThrMetGlyAlaAlaSerMetThrLeuThrVal
 30 GlnAlaArgGlnLeuLeuSerGlyIleValGlnGlnGlnAsnAsnLeuLeuArg
 AlaIleGluAlaGlnGlnHisLeuLeuGlnLeuThrValTrpGlyIleLysGln
 LeuGlnAlaArgIleLeuAlaValGluArgTyrLeuLysAspGlnGlnLeuLeu
 35 GlyIleTrpGlyCysSerGlyLysLeuIleCysThrThrAlaValProTrpAsn
 AlaSerTrpSerAsnLysSerLeuGluGlnIleTrpAsnAsnMetThrTrpMet
 40 GluTrpAspArgGluIleAsnAsnTyrThr

4. A protein having the amino-acid sequence

Met Val Trp Lys Glu Ala Thr Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr
 Asp Thr Glu Val His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro
 5 Asn Pro Gln Glu Val Val Leu Val Asn Val Thr Glu Asn Phe Asn Met Trp Lys
 Asn Asp Met Val Glu Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Gln Ser
 Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Ser Leu Lys Cys Thr Asp
 10 Leu Lys Asn Asp Thr Asn Thr Asn Ser Ser Ser Gly Arg Met Ile Met Glu Lys
 Gly Glu Ile Lys Asn Cys Ser Phe Asn Ile Ser Thr Ser Ile Arg Gly Lys Val
 15 Gln Lys Glu Tyr Ala Phe Phe Tyr Lys Leu Asp Ile Ile Pro Ile Asp Asn Asp
 Thr Thr Ser Tyr Thr Leu Thr Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys
 Pro Lys Val Ser Phe Glu Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Phe
 20 Ala Ile Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Thr Gly Pro Cys Thr Asn
 Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val Val Ser Thr Gln Leu
 25 Leu Leu Asn Gly Ser Leu Ala Glu Glu Glu Val Val Ile Arg Ser Ala Asn Phe
 Thr Asp Asn Ala Lys Thr Ile Ile Val Gln Leu Asn Gln Ser Val Glu Ile Asn
 Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg Gly Pro
 30 Gly Arg Ala Phe Val Thr Ile Gly Lys Ile Gly Asn Met Arg Gln Ala His Cys
 Asn Ile Ser Arg Ala Lys Trp Asn Asn Thr Leu Lys Gln Ile Asp Ser Lys Leu
 35 Arg Glu Gln Phe Gly Asn Asn Lys Thr Ile Ile Phe Lys Gln Ser Ser Gly Gly
 Asp Pro Glu Ile Val Thr His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr Cys
 Asn Ser Thr Gln Leu Phe Asn Ser Thr Trp Phe Asn Ser Thr Trp Ser Thr Lys
 40 Gly Ser Asn Asn Thr Glu Gly Ser Asp Thr Ile Thr Leu Pro Cys Arg Ile Lys
 Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala Pro Pro Ile
 45 Ser Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp
 Gly Gly Asn Ser Asn Asn Glu Ser Glu Ile Phe Arg Pro Gly Gly Gly Asp Met
 50 Arg Asp Asn Trp Arg Ser Glu Leu Tyr Lys Tyr Lys Val Val Lys Ile Glu Pro

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LeuGlyValAlaProThrLysAlaLysArgArgValValGlnArgGluLysArg
 AlaValGlyIleGlyAlaLeuPheLeuGlyPheLeuGlyAlaAlaGlySerThr
 5 MetGlyAlaAlaSerMetThrLeuThrValGlnAlaArgGlnLeuLeuSerGly
 IleValGlnGlnGlnAsnAsnLeuLeuArgAlaIleGluAlaGlnGlnHisLeu
 10 LeuGlnLeuThrValTrpGlyIleLysGlnLeuGlnAlaArgIleLeuAlaVal
 GluArgTyrLeuLysAspGlnGlnLeuLeuGlyIleTrpGlyCysSerGlyLys
 LeuIleCysThrThrAlaValProTrpAsnAlaSerTrpSerAsnLysSerLeu
 15 GluGlnIleTrpAsnAsnMetThrTrpMetGluTrpAspArgGluIleAsnAsn
 TyrThr

- 20 5. A protein according to any preceding claim, which does not have the N-terminal methionine.
6. DNA encoding a protein according to any preceding claim.
7. A recombinant DNA transfer vector comprising DNA according to claim 6.
8. A vector according to claim 7, which is plasmid pAPB1 or plasmid pd2PB1.
9. An immunochemical assay for detecting or quantifying antibody against HIV in a liquid, which
- 25 comprises employing a protein according to any of claims 1 to 5.
10. An immunoadsorbent, suitable for use in a solid phase immunochemical assay for antibody against HIV, comprising a solid phase to which is attached a protein according to any of claims 1 to 5.
11. An immunoadsorbent according to claim 10, further comprising a post-coat of animal protein,
12. A method of detecting antibody against HIV in a liquid, which comprises the steps of:
 - 30 (a) incubating the liquid with an immunoadsorbent according to claim 10 or claim 11, under conditions which allow the antibody (if present) in the liquid to bind to the immunoadsorbent;
 - (b) separating the immunoadsorbent from the liquid;
 - and
 - (c) determining the presence of antibody bound to the immunoadsorbent.
- 35 13. A method according to claim 12, wherein step (c) comprises incubating the immunoadsorbent with a labelled antibody against antigen of the species from which the liquid is derived; separating the immunoadsorbent from the labelled antibody; and detecting the label associated with the immunoadsorbent.
14. A method according to claim 13, wherein the labelled antibody is a labelled protein according to any
- 40 of claims 1 to 5, or labelled protein A.
15. A method according to claim 13, wherein the liquid is a human serum or plasma sample; the immunoadsorbent comprises a bead coated with the protein; and the labelled antibody is labelled anti-(human IgG) antibody.
16. A method according to claim 15, wherein the anti-(human IgG) antibody is an animal (e.g. goat)
- 45 antibody and the serum or plasma sample is diluted with normal serum from an animal of the same species.
17. A kit for use in detecting antibody against HIV, as in any of claims 12 to 16, the kit comprising:
 - (a) an immunoadsorbent according to claim 10 or claim 11;
 - (b) labelled HIV antibody; and
 - 50 (c) means for detecting the label.
18. A protein according to any of claims 1 to 5, for stimulating a lymphocyte proliferative response in humans.
19. A vaccine composition comprising one or more proteins having the antigenic properties of a protein
- 55 according to any of claims 1 to 5.

Fig. 1

ATGGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
AACCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTAACATGTGGAAA
AATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGC
CTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGAT
TTGAAGAATGATACTAATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAA
GGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAGCATAAGAGGTAAGGTG
CAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGAT
ACTACCAGCTATACGTTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGT
CCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
GCGATTCTAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTG
CTGTTAAATGGCAGTCTGGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTTC
ACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAAT
TGTACAAGACCCAAACAATAACAAGAAAAAGTATCCGTATCCAGAGAGGACCA
GGGAGAGCATTGTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGT
AACATTAGTAGAGCAAAATGGAATAACACTTTAAACAGATAGATAGCAAATTA
AGAGAACAATTTGGAAATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGG
GACCCAGAAATTGTAACGCACAGTTTTTAATTGTGGAGGGGAATTTTTCTACTGT
AATTCAACACAACCTGTTTAAATAGTACTTGGTTTAAATAGTACTTGGAGTACTAAA
GGGTCAAATAACACTGAAGGAAGTGACACAATCACCTCCCATGCAGAATAAAA
CAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCCCTCCCATC
AGTGGACAAATTAGATGTTTCATCAAATATTACAGGGCTGCTATTAACAAGAGAT
GGTGGTAATAGCAACAATGAGTCC

Fig. 2

ATGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAA
AGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTGTGTTACAATAGGAAAAATA
GGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAAATGGAATAACACT
TTAAACAGATAGATAGCAAATTAAGAGAACAATTTGGAAATAATAAAACAATA
ATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAACGCACAGTTTTAAT
TGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACGTGTTAATAGTACTTGG
TTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGACACA
ATCACCTCCCATGCAGAATAAAACAAATTATAACATGTGGCAGGAAGTAGGA
AAAGCAATGTATGCCCCTCCCATCAGTGGACAAATTAGATGTTTCATCAAATATT
ACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCC

Fig. 3

ATGCTGAACCAATCTGTAGAAATTAATTGTACAAGACCCAACAACAATACAAGAAAA
AGTATCCGTATCCAGAGAGGACCAGGGAGAGCATTGTGTTACAATAGGAAAAATA
GGAAATATGAGACAAGCACATTGTAACATTAGTAGAGCAAATGGAATAACACT
TTAAACAGATAGATAGCAAATTAAGAGAACAAATTTGGAAATAATAAAACAATA
ATCTTTAAGCAGTCCTCAGGAGGGGACCCAGAAATTGTAAACGCACAGTTTTAAT
TGTGGAGGGGAATTTTTCTACTGTAATTCAACACAACCTGTTTAATAGTACTTGG
TTTAATAGTACTTGGAGTACTAAAGGGTCAAATAACACTGAAGGAAGTGACACA
ATCACCTCCCATGCAGAATAAAACAAATTATAAACATGTGGCAGGAAGTAGGA
AAAGCAATGTATGCCCTCCCATCAGTGGACAAATTAGATGTTTCATCAAATATT
ACAGGGCTGCTATTAACAAGAGATGGTGGTAATAGCAACAATGAGTCCGAGATC
TTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATTATATAAA
TATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGAGA
AGAGTGGTGCAGAGAGAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCCTTGGG
TTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTA
CAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAAATTTGCTGAGG
GCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAG
CTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTG
GGGATTTGGGGTTGCTCTGGAAACTCATTTGCACCACTGCTGTGCCTTGGAAAT
GCTAGTTGGAGTAATAAATCTCTGGAACAGATTTGGAATAACATGACCTGGATG
GAGTGGGACAGAGAAATTAACAATTACACA

Fig. 4

ATGGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATAT
GATACAGAGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCC
AACCACACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAA
AATGACATGGTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGC
CTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTTAGTTTAAAGTGCACTGAT
TTGAAGAATGATACTAATACCAATAGTAGTAGCGGGAGAATGATAATGGAGAAA
GGAGAGATAAAAACTGCTCTTTCAATATCAGCACAAAGCATAAGAGGTAAGGTG
CAGAAAGAATATGCATTTTTTTATAAACTTGATATAATACCAATAGATAATGAT
ACTACCAGCTATACGTTGACAAGTTGTAACACCTCAGTCATTACACAGGCCTGT
CCAAAGGTATCCTTTGAGCCAATTCCCATACATTATTGTGCCCCGGCTGGTTTT
GCGATTCTAAAATGTAATAATAAGACGTTCAATGGAACAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTCAACTG
CTGTTAAATGGCAGTCTGGCAGAAGAAGAGGTAGTAATTAGATCTGCCAATTTT
ACAGACAATGCTAAAACCATAATAGTACAGCTGAACCAATCTGTAGAAATTAAT
TGTACAAGACCCAACAACAATACAAGAAAAAGTATCCGTATCCAGAGAGGACCA
GGGAGAGCATTGTGTTACAATAGGAAAAATAGGAAATATGAGACAAGCACATTGT
AACATTAGTAGAGCAAAATGGAATAACACTTTAAACAGATAGATAGCAAATTA
AGAGAACAATTTGGAATAATAAAACAATAATCTTTAAGCAGTCCTCAGGAGGG
GACCCAGAAATTGTAACGCACAGTTTTAATTGTGGAGGGGAATTTTTCTACTGT
AATTCAACACAACCTGTTTAATAGTACTTGGTTTAATAGTACTTGGAGTACTAAA
GGGTCAAATAACACTGAAGGAAGTGACACAATCACCTCCCATGCAGAATAAAA
CAAATTATAAACATGTGGCAGGAAGTAGGAAAAGCAATGTATGCCCTCCCATC
AGTGGACAAATTAGATGTTTCATCAAATATTACAGGGCTGCTATTAAACAAGAGAT

Fig.4 (cont.)

GGTGGTAATAGCAACAATGAGTCCGAGATCTTCAGACCTGGAGGAGGAGATATG
AGGGACAATTGGAGAAGTGAATTATATAAATATAAAGTAGTAAAAATTGAACCA
TTAGGAGTAGCACCCACCAAGGCCAAAGAGAAGAGTGGTGCAGAGAGAAAAAGA
GCAGTGGGAATAGGAGCTTTGTTCCCTTGGGTTCTTGGGAGCAGCAGGAAGCACT
ATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGT
ATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTG
TTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTG
GAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTGTTGGGGTTGCTCTGGAAAA
CTCATTGTCACCACTGCTGTGCCTTGGGAATGCTAGTTGGAGTAATAAATCTCTG
GAACAGATTTGGAATAACATGACCTGGATGGAGTGGGACAGAGAAATTAACAAT
TACACA

olig: AGGAGTCCCTTATGCTGAACCAATCTGTA

Fig. 5

← env →
AACATGAGTCCGAGATCGTGGACAAGCTTCCCGGAGCTCGAATTCTTGAAGACGAAAGGGCCT...

olig: AACATGAGTCCGAGATCTGAAGACGAAAGGGCCTCGTG

Fig. 6



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(54) **Recombinant polypeptides and their uses, including assay for AIDS virus.**

(57) The HIV portions of 4 proteins are useful for the diagnosis of, and in preparing vaccines for, the HIV virus.

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 84, no. 14, July 1987; K. KROHN et al.: "Specific cellular immune response and neutralizing antibodies in goats immunized with native or recombinant envelope proteins derived from human T-lymphotropic virus type IIb and in human immunodeficiency virus-infected men" * Whole document *	1	C 07 K 13/00 C 12 N 15/00 G 01 N 33/569 A 61 K 37/02 A 61 K 39/21 C 12 P 21/02
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CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			